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CUTANEOUS NERVE CHANGES IN ZOSTER*

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Although the principal clinical and histopathologic features of herpes zoster are caused by the infection of epidermal cells by the varicella-zoster virus, the dermatomal distribution of the lesions, the pathologic involvement of the sensory ganglia, and the characteristic occurrence of pain clearly emphasize the associated underlying infection of nerve tissues. The present viewpoint is that a latent virus infection is set up principally in the spinal and cranial sensory ganglia as a result of hematogenous dissemination during the initial varicella infection and is activated in later life so that the virus spreads down the peripheral nerve into the skin (1, 2).

There have been relatively few studies of the frequency and extent of the loss of axons in the peripheral nerves or skin of affected dermatomes in patients with zoster, as most reports have dealt only with isolated observations in postmortem examinations (3–13). Ebert (8) attempted to evaluate the extent of cutaneous nerve injury in the skin of affected dermatomes in a systematic fashion. He used a silver method for staining cross sections of skin biopsy specimens in 12 patients and observed a variable reduction in the number of axons in the larger nerves of the middle and lower dermis in all 5 patients who had had the eruption for at least 14 days but not in the axons of patients whose cutaneous lesions were of shorter duration. Unfortunately, control biopsies of the skin of unaffected dermatomes were not done and, except in two instances, biopsy specimens were taken at sites of inflammatory lesions or scars. There also were no clinical data given concerning the relationship of pain to the nerve loss. More recently, Lourie and King (13) have confirmed Ebert's findings with silver methods but do not give any clinical data or the number of patients with zoster studied.

The extent of nerve-fiber loss in the skin and also its relationship to pain have not been determined. That zoster may occur without significant loss of nerve fibers is suggested by the common occurrence of mild zoster without significant symptoms and by the rare reports of cases in which only slight or no involvement of dorsal root ganglia was found (3, 7, 14–16). On the other hand, the presence of hypo-esthesia in the skin and reports of postmortem examination showing severe necrosis and even cyst formation in the spinal ganglia indicate that extensive or total loss of sensory ganglion cells has occurred. In this regard, Noordenbos (17) has observed that postherpetic neuralgia is seen only among patients with significant sensory loss, and Lourie and King (13) have reported hyperpathia only when hypo-esthesia is present.

The purpose of the present study was to determine the frequency and extent of nerve changes in the skin of affected dermatomes of patients with zoster. We sought objective means of evaluating loss of nerve fibers because sensory examination is the most difficult and least reliable part of the neurologic examination. Previous studies had shown the practicality of studying large areas of nerve pathways in the skin by means of whole mount preparations stained by the acetylcholinesterase reaction after removal of the epidermis with sodium bromide (18). Whole mount cholinesterase techniques for nerve staining have also been found practicable in the study of tendons, fascia, joint capsules, ligaments, and periosteum (19, 20). We felt that this method would be accurate, since the innervation of relatively large areas of tissue could be assessed and compared relatively easily and since the stain demonstrates nerve tissue clearly. Later vertical cross sections stained similarly were also prepared to see whether additional information could be obtained. It was determined that the vertical sections were superior for demonstrating residual nerve fibers in the more severely denervated specimens.

In addition, the function of the sensory nerves in the skin of the affected dermatomes was measured by the presence of the axon

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flare after the intradermal injection of histamine.

MATERIALS AND METHODS

We studied 23 patients (11 men and 12 women) who had active or recently healed lesions of zoster or postherpetic neuralgia of variable duration and whom we had seen during 15 months. Their ages ranged from 42 to 81 years (average 64, median 65). The right side of the body was affected 12 times and the left side 11 times. The dermatome distribution of the lesions showed involvement of the ophthalmic branch of the trigeminal nerve in 3 patients, cervical nerves in 4, thoracic nerves in 12, and lumbar nerves in 4. Four patients had lymphoma and two additional patients had had a carcinoma of the stomach or uterus excised here surgically 1 and 5 years previously but were well otherwise. In no case was neoplasm present in or near the segments affected with zoster, either in the skin or about the vertebral tissues.

All the patients had pain, but special note was made of the severity and persistence of the pain, which was graded as mild, moderate, or severe. Only that pain which was not relieved for the most part by aspirin or propoxyphene hydrochloride (Darvon) was graded as severe.

Punch biopsy specimens 4 or 5 mm in diameter were obtained in all 23 patients from the normal-appearing skin present between the inflammatory vesicular plaques or between the areas of scarring and pigmentation in the affected dermatomes and from symmetrical sites in the unaffected corresponding dermatomes of the contralateral side. The tissue was frozen immediately and stored as such until ready for staining, which was usually done within a few days. The paired skin biopsy specimens in the first 13 patients seen were processed as whole mount preparations which were stained by the acetylcholinesterase reaction after removal of the epidermis with sodium bromide as previously reported (18). The specimens from affected and uninvolved dermatomes were stained simultaneously as matched pairs for technical control. Each author then viewed independently the stained whole mounts without knowing the affected side. The amount of nerve tissue was judged to be either approximately equal to or decreased from that of the opposite side. Later the slides judged to have decreased nerve staining were reviewed again and a third category was set forth to indicate when the nerve loss had been unusually severe.

The paired biopsy specimens from the remaining 10 patients were cut vertically into sections 50 or 70 μ thick and were stained by a modified Gomori acetylcholinesterase reaction (21). Approximately 18 sections per biopsy specimen were prepared, with 6 sections per slide. Two pairs of biopsy specimens were not frozen immediately, resulting in a marked loss of enzyme reactivity, and were discarded. The amount of nerve tissue stained was evaluated as before.

The functional integrity of the sensory afferent nerves in the affected and paired normal dermatomes was then determined by the presence or absence of the histamine flare (22). An intradermal injection of 0.1 to 0.2 ml of 1:100,000 solution of histamine phosphate was made into the normal-appearing skin of the affected dermatome and into a symmetrical site in the paired unaffected dermatome. The size of the axon flare was measured in two diameters and a significant change was considered to have been present only if the axon flare was completely absent or almost so in the affected dermatome but floridly present in the contralateral site. The skin of the trunk was the best site for eliciting axon flares. The axon response may be poorly defined or absent normally in the skin of the face or extremities, so that comparison of the axon flares between normal and affected sides is very important.

RESULTS

The dermal nerve network, as stained by the acetylcholinesterase method, was found to be significantly reduced in the normal-appearing skin of the dermatomes affected with zoster in 11 of 13 patients from whom biopsy specimens were evaluated independently as whole mount preparations by each of us. In five of these patients the loss of nerve fibers was especially severe. Two patients had approximately equal amounts of nerve fiber staining in affected and unaffected sides. Of the eight patients whose skin biopsy specimens were sectioned vertically and stained by the acetylcholinesterase method, four had a decrease in number of dermal nerves and one of these was judged to have an unusually severe loss; the other four patients had approximately equal numbers of nerves in affected and unaffected sides.

Although the results of study of the biopsy specimens by vertical sectioning were similar to those obtained with whole mounts, it was our general impression that gross differences in the amount of nerve fiber staining present were easier to ascertain in the whole mounts of dermis because larger areas of tissue were examined, yet only two sections of tissue needed to be compared. In the vertical-section method, several sections from each biopsy specimen needed to be studied in order to determine the average concentration and distribution of nerve fibrils, and a final opinion depended on the fusion of results from looking at several microscopic sections. However, when severe loss of nerve fibrils had occurred, more detailed observations could be made on the clear,

thin, vertical sections than on the opaque, thick, whole mounts.

Among the 23 patients with zoster in this study, normal axon flares in response to the intradermal injection of a 1:100,000 solution of histamine phosphate were seen in the affected dermatomes of 13 patients and, in addition, in patients 17 and 23 (Table I) whose biopsy specimens were discarded because of inadequate freezing. The remaining eight patients did not have an axon flare in the affected dermatome.

The patients with normal axon flares had pain that was less severe and of shorter duration than in the other patients and which usually subsided by the time the skin eruption resolved. In three cases, severe pain occurred initially but decreased greatly or resolved with healing of the cutaneous lesions so that analgesic medication was not required subsequently.

The biopsy specimens of skin from affected dermatomes in patients with normal axon flares were generally normal or showed only mild to moderate loss of nerve fiber staining. Of the whole mount preparations, two showed no loss, four moderate loss, and one severe loss of nerve fibers. In the biopsy specimens studied by vertical section, no differences in nerve staining between specimens from affected and those from unaffected dermatomes were observed in four patients, while two patients showed moderately decreased numbers of nerve fibers.

Severe persistent pain was characteristic of the group without axon flares, though in patient 2 the pain decreased greatly after 6 weeks. The most severe loss of nerve fibers was seen in the skin specimens from affected dermatomes in patients who had absence or decrease of axon flares: five patients had severe loss while three had only moderate loss of nerve fibers.

The earliest loss of nerve staining was detected on the day of onset of the eruption in patients 1 and 21, but the pain had already been present in the dermatome for 9 days in the latter, while in the former the pain had begun a few hours before the eruption had been noted. The longest time that loss of nerve staining persisted was 480 days. Early loss of nerve staining could not be related to an increased severity or persistence of pain.

DISCUSSION

A qualitative comparison of nerve fibrils stained by the acetylcholinesterase method in paired skin biopsy specimens from affected and unaffected dermatomes in patients with zoster is possible but gives only a rough quantitative estimate of nerve changes. The extent of the nerve network in the dermis varies normally from region to region (Figs. 1 and 2) and even from person to person, making controlled observations at symmetrical sites imperative in each patient. Nevertheless, our results have confirmed and extended the previous observations of axonal loss in the skin which had been inferred frequently but rarely observed directly (8, 9, 13). We have shown that partial denervation is a common occurrence in zoster, even though it may not be detectable clinically, but it is rarely severe enough to cause anesthesia. A severe loss of nerve-fiber staining was associated with severe pain and increased sensory loss in the skin—observations that are similar to those of Noordenbos (17). In addition, the loss of nerve-fibril staining in the skin of affected dermatomes which we observed on the first day of the eruption in two cases confirms the early involvement of the primary sensory neuron in zoster and suggests that active infection of nerve tissues has preceded involvement of the skin. Pain has been reported to precede the onset of cutaneous lesions not uncommonly, even by as much as 12 days (23), and a form of zoster without skin lesions has also been postulated (24).

Our findings suggest that absence of the axon flare can best be explained on the basis of a marked functional decrease in the number of sensory afferent nerves remaining in the skin. Since complete anesthesia was not present in any of the patients who had absence of axon flares associated with severe herpetic neuralgia, we have postulated that a certain minimal concentration of sensory afferent nerves is needed to elicit a normal axon flare. A decrease below this critical level would cause a decrease or absence of the axon flare and yet there would still be limited somatosensory perception in the skin.

The absence of an axon flare after the intradermal injection of histamine into the affected dermatome in the majority of patients with

TABLE I

Results of staining by acetylcholinesterase method

Case	Age (yr)	Sex	Segment	Neoplasm	Pain	Days of eruption before biopsy	Number of fibers on nerve staining	Histamine flare
Whole mount preparations								
1	42	M	T-12	—	Mild	1	Marked decrease	Normal
2	43	F	T-5	—	Severe pain, moderately less after 6 wk	35	Decreased	Reduced 60%; less pink
3	49	M	L-5	Retic. cell sarcoma	Severe; also motor weakness	9	Marked decrease	Absent
4	58	M	C-2 or 3	—	Moderate	3	No change	Normal
5	60	F	C-2	Hodgkin's disease	Moderate	6	Decreased	Normal
6	65	M	T-11	—	Moderate discomfort persisted, relieved by aspirin	60	Decreased	Normal
7	66	M	T-11	—	Moderate	14 (21)*	Decreased	Normal
8	66	F	L-1	—	Severe	28	Marked decrease	Absent
9	69	F	T-7	—	Initially severe pain, relieved after 3 days	8	Decreased	Normal
10	70	M	T-2 or 3; also disseminate vesicles	Lymphoblastic lymphocytic lymphoma	Severe	35	Marked decrease	Absent
11	71	F	Ophthalmic branch, 5th nerve	—	Severe	480	Decreased	Absent
12	74	M	T-9	—	Moderate	4	No change	Normal
13	81	M	Ophthalmic branch, 5th nerve	—	Severe	22	Marked decrease	Absent
Vertical section preparations								
14	47	F	C-5 or 6	—	Severe initially, cleared in 2 wk	12	No change	Normal
15	52	F	Ophthalmic branch, 5th nerve	—	Mild	10	No change	Normal
16	58	M	L-2	—	Severe	99	Marked decrease	Absent
17	63	F	L-2	—	Severe initially, cleared on healing of rash	Discarded		Normal
18	64	F	T-12	—	Moderate	10	Decreased	Normal
19	65	M	T-9	Carcinoma, stomach	Moderate	26	No change	Normal
20	73	M	T-6	—	Severe	480	Decreased	Absent
21	73	F	T-8	Adenocarc., uterus	Moderate	1 (9)*	Decreased	Normal
22	76	F	T-4	—	Moderate	3 (7)*	No change	Normal
23	76	F	C-7	Hodgkin's disease	Moderate	Discarded		Normal

* Days after onset of pain are given in parentheses.

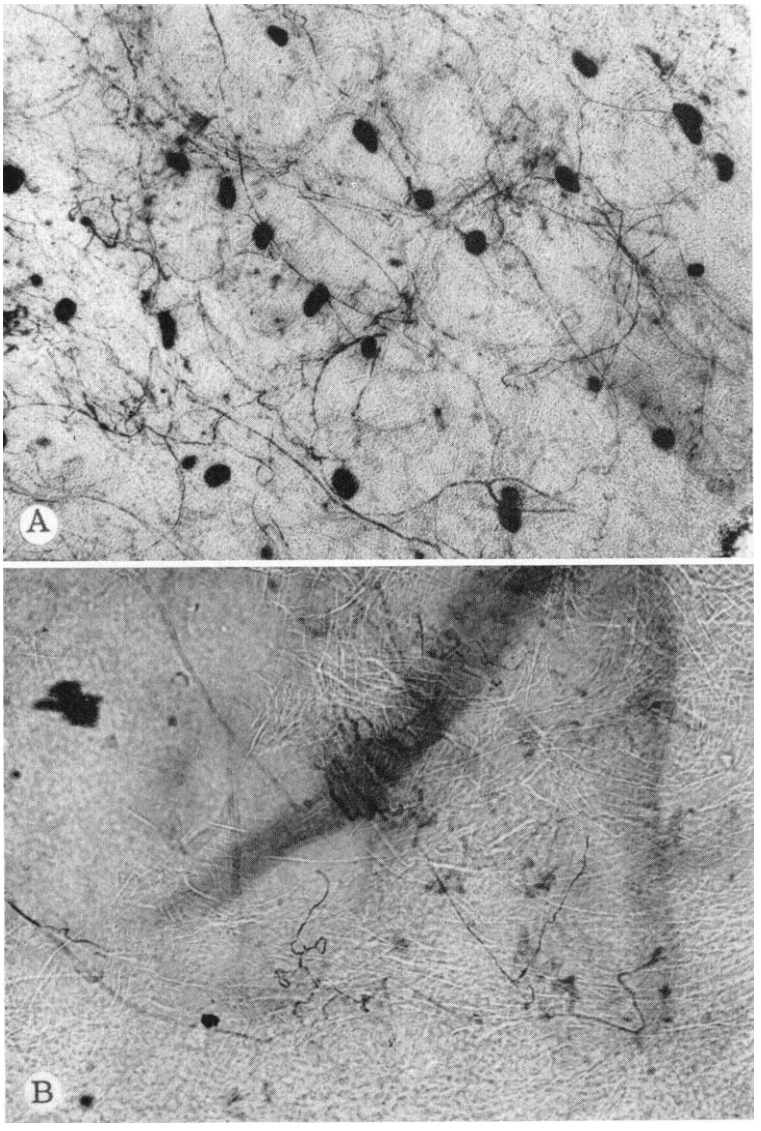

FIG. 1. Acetylcholinesterase-positive nerves in normal skin. *A*, Fingertip. Meissner's corpuscles also stained. (Whole mount; × 50.) *B*, Thorax. Arrectores pilorum also positive. (Whole mount; × 170.)

zoster in whom pain was a severe complication suggested that the histamine test is a simple, useful prognostic aid in predicting the occurrence of the major complication of zoster, severe herpetic neuralgia. On the other hand, a normal axon flare was usually associated with pain of less severity and shorter duration.

Weddell (9) examined sections of intercostal nerves obtained at autopsy from normal and affected dermatomes in four patients who had had postherpetic neuralgia. Histograms showed that the numbers of large medulated nerve fibers were greatly reduced, while the numbers of small medulated and unmyelinated fibers were significantly increased. He favored the view that the loss of nerve fibers resulted from random reduction in the number of dorsal root ganglion cells and stated that the affected skin in these cases of postherpetic neuralgia was sparsely innervated with fine

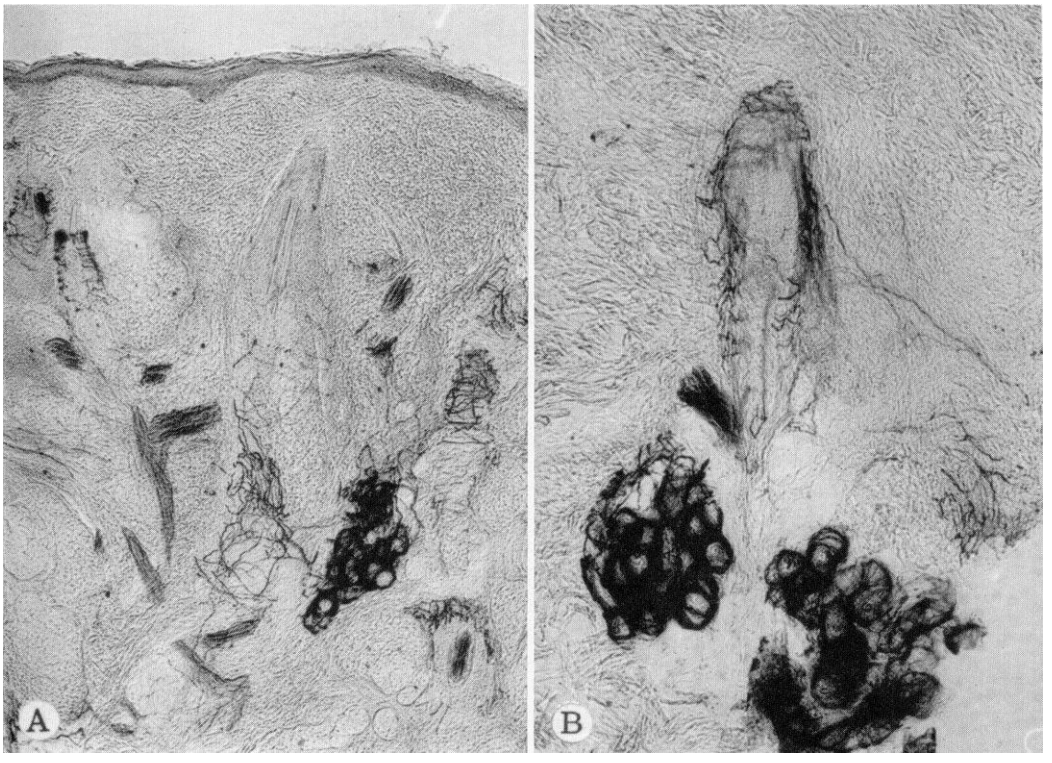

FIG. 2. Acetylcholinesterase-positive nerves about hair follicles, sweat glands, and free in normal skin. *A,* Forehead. (Vertical section; × 50.) *B,* Upper back. (Vertical section; × 75.)

axons served by nerve bundles containing a preponderance of fine nerve fibers of slow conducting velocity.

Zacks and associates (11) studied by conventional light microscopy, phase contrast microscopy, and electron microscopy, excised segments of cutaneous nerves obtained from the affected skin of four patients of whom two had postherpetic neuralgia at varying intervals from 8 days to 24 months after onset of the rash. In the nerves of all four patients they found abnormalities, principally degeneration of the larger fibers including loss of axon cylinders and degeneration of myelin. The smallest nerve fibers were intact but these authors were of the opinion that these fibers were also eventually lost. Fibrous proliferation and nearly complete absence of nerve fibers were late occurrences in the nerve segments examined. The presence and extent of neural fibrosis could not be related to the development of postherpetic neuralgia. Many more studies of this kind are needed to establish the variations, significance, and relationship of the specific nerve changes to the various clinical syndromes of zoster.

SUMMARY

The cutaneous nerves in the normal-appearing skin of affected and uninvolved symmetrical dermatomes were studied in 23 patients with zoster by means of skin biopsy specimens stained according to the acetylcholinesterase method and by means of intradermal histamine tests. A qualitative comparison of nerve fibrils stained with acetylcholinesterase in paired skin biopsy specimens processed either as whole amounts of dermis or as thick vertical sections gave a rough quantitative estimate of nerve changes and showed that partial denervation is a common occurrence in the skin of the affected dermatome in patients with zoster. The dermal nerve network was significantly reduced in the skin of the affected dermatomes in 15 patients, and in 6 of these the loss of nerves was judged to be unusually severe.

Loss of nerve fibril staining was seen on the first day of the eruption and persisted as long as 480 days. The early involvement of nerves and the persistence of denervation in the skin of affected dermatomes in patients with postherpetic neuralgia support the theory that the sensory ganglion is the primary focus of involvement in zoster.

Normal axon flares elicited by the intradermal injection of 0.1 ml of 1:100,000 solution of histamine phosphate in the affected dermatomes were present in 15 patients and were absent or greatly diminished in 8. The patients with normal axon flares had pain that was less severe and of shorter duration, though 3 patients initially had severe pain that subsequently decreased greatly or resolved with healing of the eruption. On the other hand, severe pain during the acute phases of zoster and postherpetic neuralgia commonly occurred in the patients with absence or decrease of axon flares. The most severe loss of nerve fibers was usually seen in the skin biopsy specimens from affected dermatomes in those patients who did not have axon flares.

The histamine test is proposed as a simple and useful prognostic sign that will frequently predict the occurrence of severe herpetic neuralgia, absence of the axon flare indicating a course with more severe and prolonged pain, and a normal axon flare indicating pain which will be less severe and of shorter duration.

REFERENCES

1. Hope-Simpson, R. E.: The nature of herpes zoster: A long-term study and a new hypothesis. Proc. Roy. Soc. Med., *58:* 9, 1965.
2. Gold, E.: Serologic and virus-isolation studies of patients with varicella or herpes-zoster infection. New Eng. J. Med., *274:* 181, 1966.
3. Head, H. and Campbell, A. W.: The pathology of herpes zoster and its bearing on sensory localisation. Brain, *23:* 353, 1900.
4. Lhermitte, J. and Nicolas, V.: Les lésions spinales du zona: La myélite zostérienne. Rev. Neurol. (Paris), *1:* 361, 1924.
5. Denny-Brown, D., Adams, R. D. and Fitzgerald, P. J.: Pathologic features of herpes zoster: A note on "geniculate herpes." Arch. Neurol. Psychiat., *51:* 216, 1944.
6. Goodbody, R. A.: The pathology of acute herpes zoster ophthalmicus. J. Path. Bact., *65:* 221, 1953.
7. Barontini, F.: Sulle alterazioni istopatologiche del sistema nervoso nell' herpes zoster. Riv. Pat. Nerv. Ment., *78:* 304, 1957.
8. Ebert, M. H.: Histologic changes in sensory nerves of the skin in herpes zoster. Arch. Derm., *60:* 641, 1949.
9. Weddell, G.: Cited by Noordenbos, W. (17).
10. Marinesco, G.: Nouvelles contributions à la pathogénie et à la physiologie du zona. Ann. Derm. Syph. (Paris), *62:* 641, 1931.
11. Zacks, S. I., Langfitt, T. W. and Elliott, F. A.: Herpetic neuritis: A light and electron microscopic study. Neurology (Minneap.), *14:* 744, 1964.
12. Blackley, B., Friedmann, I. and Wright, I.: Herpes zoster auris associated with facial nerve palsy and auditory nerve symptoms: A case report with histopathological findings. Acta Otolaryng. (Stockholm), *63:* 533, 1967.
13. Lourie, H. and King, R. B.: Sensory and neurohistological correlates of cutaneous hyperpathia. Arch. Neurol. (Chicago), *14:* 313, 1966.
14. Wohlwill, F.: Zur pathologischen Anatomie des Nervensystems beim Herpes Zoster (auf Grund von zehn Sektionsfällen). Z. Ges. Neurol. Psychiat., *89:* 171, 1924.
15. Nieuwenhuijse, P.: Die pathologische-anatomische Untersuchung eines Falles von Herpes Zoster. Z. Ges. Neurol. Psychiat., *22:* 45, 1914.
16. Zumbusch, L.: Uber Herpes Zoster generalisatus mit Rückenmarks-veränderungen. Arch. Derm. Syph., *118:* 823, 1913.
17. Noordenbos, W.: *Pain: Problems Pertaining to the Transmission of Nerve Impulses Which Give Rise to Pain: Preliminary Statement.* Elsevier Press, Inc., Amsterdam, 1959.
18. Winkelmann, R. K., Wolff, K. and Pierce, J.: Removal of epidermis with sodium bromide for cholinesterase staining of dermal nerves. Stain. Tech., *42:* 214, 1967.
19. Lipscomb, P. R. and Winkelmann, R. K.: A whole-mount cholinesterase technic for study of innervation of the deep structures of the limbs. J. Invest. Derm., *37:* 481, 1961.
20. Jackson, H. C., Winkelmann, R. K. and Bickel, W. H.: Nerve endings in the human lumbar spinal column and related structures. J. Bone Joint Surg. [Amer.], *48:* 1272, 1966.
21. Winkelmann, R. K. and Schmit, R. W.: Cholinesterases in human skin. Arch. Derm., *80:* 543, 1959.
22. Rothman, S.: *Physiology and Biochemistry of the Skin.* University of Chicago Press, Chicago, Ill., 1954.
23. Carter, A. B.: Investigation into the effects of aureomycin and chloramphenicol in herpes zoster. Brit. Med. J., *1:* 987, 1951.
24. Lewis, G. W.: Zoster sine herpete. Brit. Med. J., *2:* 418, 1958.